

Application No. 10/706,701  
Amendment dated February 11, 2008  
Reply to Office Action of January 7, 2008

Docket No.: NY-ROCHE 203-US

RECEIVED  
CENTRAL FAX CENTER

FEB 11 2008

AMENDMENTS TO THE CLAIMS

1. (Currently amended) A method of treating disturbances in iron distribution in a patient suffering from heart disease comprising administering to a patient suffering from heart disease and disturbances in iron distribution an therapeutically effective amount of human erythropoietin protein having the amino acid sequence of SEQ ID NO: 1 sufficient to alleviate said disturbances in iron distribution without administering iron.
2. (Original) The method of claim 1, wherein the patient is suffering from heart insufficiency.
3. (Canceled)
4. (Canceled)
5. (Previously presented) A method of treating disturbances in iron distribution in a patient suffering from heart disease comprising administering a therapeutically effective amount of human erythropoietin protein having the amino acid sequence of SEQ ID NO: 1 modified by the addition of up to three glycosylation sites, without administering iron, wherein the modification is selected from the group consisting of:
  - Asn<sup>30</sup>Thr<sup>32</sup>;
  - Asn<sup>51</sup>Thr<sup>53</sup>;
  - Asn<sup>57</sup>Thr<sup>59</sup>;
  - Asn<sup>69</sup>;
  - Asn<sup>69</sup>Thr<sup>71</sup>;
  - Ser<sup>68</sup>Asn<sup>69</sup>Thr<sup>71</sup>;

Application No. 10/706,701  
 Amendment dated February 11, 2008  
 Reply to Office Action of January 7, 2008

Docket No.: NY-ROCHE 203-US

Val<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;  
 Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;  
 Ser<sup>87</sup>Asn<sup>88</sup>Gly<sup>89</sup>Thr<sup>90</sup>; (SEQ ID NO: 2);  
 Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>Thr<sup>92</sup>;  
 Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>Ala<sup>162</sup>;  
 Asn<sup>69</sup>Thr<sup>71</sup>Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;  
 Asn<sup>30</sup>Thr<sup>32</sup>Val<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup>;  
 Asn<sup>89</sup>Ile<sup>90</sup>Thr<sup>91</sup>;  
 Ser<sup>87</sup>Asn<sup>89</sup>Ile<sup>90</sup>Thr<sup>91</sup>;  
 Asn<sup>136</sup>Thr<sup>138</sup>;  
 Asn<sup>138</sup>Thr<sup>140</sup>;  
 Thr<sup>125</sup>; and  
 Pro<sup>124</sup>Thr<sup>125</sup>.

6. (Currently Amended) A method of treating disturbances in iron distribution in a patient suffering from heart disease comprising administering a therapeutically effective amount of human erythropoietin protein, without administering iron, wherein the protein (EPO) is an analog of SEQ ID NO: 1, said analog is selected from the group consisting of: (a) human erythropoietin protein having the amino acid sequence, Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln (SEQ ID NO: 3), extending from the carboxy terminus; (b) the analog in (a) further comprising Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup> EPO; (c) the analog in (a) further comprising Asn<sup>30</sup>Thr<sup>32</sup>Val<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup> EPO; (d) the analog in (a) further comprising Gln<sup>24</sup>Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup> EPO; (e) the analog in (a) further comprising Gln<sup>38</sup>Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup> EPO; (f) the analog in (a) further comprising Gln<sup>83</sup>Ser<sup>87</sup>Asn<sup>88</sup>Thr<sup>90</sup> EPO and (g) darbepoetin alfa.

Application No. 10/706,701

Amendment dated February 11, 2008

Reply to Office Action of January 7, 2008

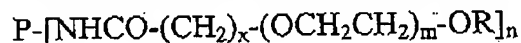
Docket No.: NY-ROCHE 203-US

7. (Original) The method of claim 1, wherein the erythropoietin protein is pegylated.

8. (Previously presented) A method of treating disturbances in iron distribution in a patient suffering from heart disease comprising administering a conjugate of human erythropoietin protein of SEQ ID NO: 1 without administering iron, wherein said conjugate comprising the erythropoietin protein of SEQ ID NO:1 having one to three free amino groups covalently linked to n poly(ethylene glycol) groups of the formula  $-\text{CO}-(\text{CH}_2)_x-(\text{OCH}_2\text{CH}_2)_m-\text{OR}$  with the  $-\text{CO}$  of each poly(ethylene glycol) group forming an amide bond with one of said amino groups; wherein R is a lower-alkyl; x is 2 or 3; m is from about 450 to about 900; n is from 1 to 3; and n and m are chosen so that the molecular weight of the conjugate minus the erythropoietin protein is from 20 kilodaltons to 100 kilodaltons.

9. (Original) The method of claim 8, wherein x is 2, m is 650 to about 750, n is 1 and R is methyl.

10. (Original) The method of claim 8 wherein the conjugate has the formula



wherein

P is the residue of the erythropoietin protein without the free amino group that forms the amide linkage;

R is lower alkyl;

x is 2 or 3;

m is from about 450 to about 900; and

n is from 1-3;

Application No. 10/706,701

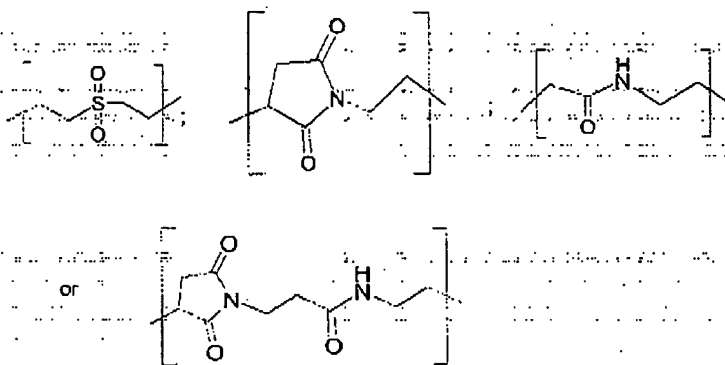
Amendment dated February 11, 2008

Reply to Office Action of January 7, 2008

Docket No.: NY-ROCHE 203-US

and wherein m and n are selected such that the molecular weight of the conjugate minus the erythropoietin protein is from about 20 kD to about 100 kD.

11. (Previously presented) A method of treating disturbances in a patient suffering from heart disease comprising administering a conjugate of human erythropoietin protein of SEQ ID NO: 1, without administering iron, wherein said conjugate comprises the erythropoietin protein having one to three free amino groups covalently linked to from one to three lower-alkoxy poly(ethylene glycol) groups, each poly(ethylene glycol) group being covalently linked to the erythropoietin protein via a linker of the formula  $-C(O)-X-S-Y-$  with the C(O) of the linker forming an amide bond with one of said amino groups, X is  $-(CH_2)_k-$  or  $-CH_2(O-CH_2-CH_2)_k-$ , k is from 1 to 10, Y is



the average molecular weight of each poly(ethylene glycol) moiety is from about 20 kilodaltons to about 40 kilodaltons, and the molecular weight of the conjugate is from about 51 kilodaltons to about 175 kilodaltons.

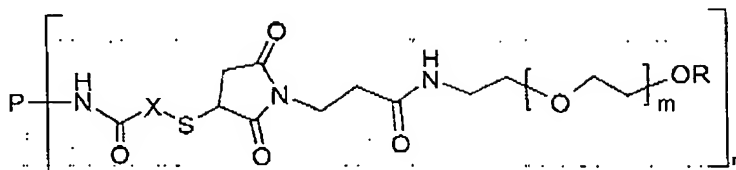
12. (Original) The method of claim 11, wherein the erythropoietin conjugate has the formula:

Application No. 10/706,701

Docket No.: NY-ROCHE 203-US

Amendment dated February 11, 2008

Reply to Office Action of January 7, 2008



wherein  $n$  is an integer from 1 to 3;  $m$  is an integer from 450 to 900;  $R$  is lower-alkyl;  $X$  is  $-(CH_2)_k-$  or  $-CH_2(O-CH_2-CH_2)_k-$ ,  $k$  is 1 to 10 and  $P$  is the residue of the erythropoietin protein without the  $n$  amino groups which form an amide linkage with  $X$ .

13. (Previously presented) The method of claim 1 wherein the amount of human erythropoietin protein administered to the patient is from about 100 U/kg to about 200 U/kg twice per week.

14. (Previously presented) The method of claim 10 wherein the amount of the human erythropoietin protein administered to the patient is about 200 U/kg once every three weeks.